tions may be important in relation to infectivity, because of the differences in the microbic flora of the two regions.

Problems of rates of and distances to evaporation, and of minimum droplet size are being investigated.

11231 P

Effect of Repeated Injections of Cobra Venom on Blood Chemistry and Morphology.

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Large doses of cobra venom were repeatedly injected in rabbits for periods varying from 2 to 21 weeks, and quantitative studies were made on the morphology and chemistry of their blood. The venom solution was usually injected 5 times a week and the dosage administered on injection varied from 5 to 10 mouse units. Inasmuch as 5 mouse units constitute the usual therapeutic dose of cobra venom for human beings and 10 mouse units are employed only in more refractory cases, the dosages given rabbits in the present study were manifestly enormous. The results obtained from 10 rabbits are reported in this investigation. Three of the rabbits were used as controls and received no cobra venom while the other 7 received the drug partly by the intravenous and partly by the intramuscular route. Rabbits of different weight were purposely selected at the beginning of the research but all were kept under the same conditions and fed with Purina Chow supplemented with fresh green vegetables. The cobra venom solution (H. W. & D.) employed was prepared in these laboratories and assayed by the authors on white mice by methods described elsewhere.[1] This solution is the same as that employed by various authors in clinical studies published in different journals.[2-6] Crude cobra venom consists largely of neurotoxin and contains relatively small quantities of cytotoxic and proteolytic constituents in striking

[1] Macht, *Proc. Nat. Acad. Sc.*, 1936, **22**, 61.

[2] Macht, *Med. Rec.*, 1936, **144**, 537.

[3] Macht, *Ann. Int. Med.*, 1938, **11**, 1824.

[4] Gayle and Williams, *Southern M. J.*, 1938, **31**, 188.

[5] Rutherford, *New England J. Med.*, 1939, **221**, 408.

[6] Black, *Southern M. J.*, 1940,

contrast to the crude venom of the *Viperidae (Crotalus, Bothrops, Agkistrodon,* and Russell's viper), which are rich in cytotoxic and blood-affecting elements but contain very small amounts of the neurotoxic substance.[7] In the preparation of the cobra venom solution employed by the authors and in the process of sterilizing it, the proteins of the crude substance are almost entirely removed and the cytotoxic and hemolytic constituents are eliminated by at least 50%. The resultant solution of cobra venom therefore consists largely of the neurotoxin which is quite stable and is responsible for the analgesic and other therapeutic properties of cobra venom. This neurotoxin, according to the latest chemical work on the subject, is not a protein but resembles the glucosides in its structure.[8]

In the present study the authors have determined the absolute number of erythrocytes and leukocytes, the differential leukocyte count and the percentage of hemoglobin in the blood. Quantitative biochemical determinations were also made on blood urea, blood urea nitrogen and blood sugar by the usual standard clinical methods (Folin). The results obtained are exhibited in Table I, which indicates the average weight of the animals, the total amount of venom injected in each and the period over which the drug was administered, in addition to the data obtained from the morphological and biochemical examination of the blood. Kidney and liver function tests were also made in these rabbits but are not mentioned in the table as they have already been discussed elsewhere in these PROCEEDINGS.[9] It will be seen that the data set forth in the tables reveals that no specific change occurred in the blood of the treated rabbits as compared with the normal controls and with each other. Certainly no characteristic injuries or pathological effects were noted in the blood examinations. The considerable anemia and high eosinophile count obtained in Rabbit No. 1 were due to the parasitic fungous infection of the ears frequently encountered in laboratory rabbits. The total amount of cobra venom injected in the rabbits varied from 45 to 990 mouse units. None of the rabbits treated with cobra venom revealed any difference in duration of coagulation time. The weight of the animals determined at the end of a period of experimentation differed but slightly from that registered at the beginning of the research. The young rabbits, of course, increased in weight as they grew older but the increment in this case was the same as in the normal controls. Further researches along the above-mentioned

[7] Noc, *Ann. Inst. Pasteur,* 1904, **18**, 387.

[8] Micheel and Bode, *Naturwissenschaften,* 1938, **26**, 298.

[9] Macht and Brooks, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 418.

TABLE I.
Effect of Cobra Venom on Morphology and Chemistry of the Blood of Rabbits.

Rabbit No.	Avg kg wt of rabbit	No. of weeks rabbit inj.	Total No. mouse units cobra venom inj.	Blood urea	Blood urea N	% of blood sugar	Red blood corpuscles	% of hemoglobin	Leukocyte count	Differential Leukocyte Count						
										Small lymphocytes	Large lymphocytes	Large mononuclears	Polymorphonuclears	Eosinophiles	Neutrophilic myelocytes	Basophiles
1	3.65	13	990	53.5	25.0	0.119	4,176,000	58	14,250	27	7	15	15	32	4	—
2	2.68	12	630	49.2	23.0	0.103	5,600,000	75	18,200	55	14	2	23	6	—	—
3	2.30	3	90	49.2	23.0	0.147	5,632,000	81	17,150	59	13	7	16	1	2	2
4	2.24	21	850	40.0	18.7	0.153	5,368,000	85	8,450	50	16	7	24	2	—	1
5	1.00	Control	0	49.2	23.0	0.111	5,056,000	75	9,600	64	6	3	25	1	—	1
6	4.52	2½	60	61.0	28.5	0.107	5,328,000	78	8,800	39	4	21	33	—	—	2
7	2.00	Control	0	38.7	18.1	0.162	5,984,000	95	10,550	68	5	4	18	3	1	1
8	1.85	7	265	45.8	21.4	0.100	5,840,000	96	14,200	53	11	7	22	3	3	1
9	4.00	Control	0	45.8	21.4	0.111	4,680,000	95	11,200	65	6	5	21	3	—	—
10	3.00	2	45	49.2	23.0	0.133	4,144,000	76	9,250	52	8	6	26	4	4	—

lines are in progress. It is interesting to note that the findings set forth in the present paper regarding rabbits injected with large doses of cobra venom over long periods of time agree with the clinical observations of Steinbrocker, McEachern, La Motta and Brooks[10] in their study on human subjects with material supplied by the senior author of the present paper. These findings agree also with the undetailed report recently published concerning the work of French investigators, who found that no change was produced in the blood sugar level of rabbits but that a rise in blood sugar of guinea pigs was effected by injections of a cobra venom preparation, regarding which details are not given.[11]

Summary. Large quantities of cobra venom were injected in a series of rabbits for periods varying from 2 to 21 weeks. Morphological and biochemical studies on the blood revealed no striking pathological change and no specific effect on the blood picture of the animals as compared with normal controls.

11232 P

Toxicity for Dogs of a Bactericidal Substance Derived from a Soil Bacillus.

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(Introduced by Rene J. Dubos.)

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Dubos[1] has described the isolation from a sporulating soil bacillus of an agent which exerts a powerful bactericidal effect *in vitro* upon Gram positive bacteria, and which if injected intraperitoneally affords protection to white mice infected by the same route with pneumococci or hemolytic streptococci. Subsequently Dubos and Cattaneo[2] reported the results of studies with a protein-free preparation of the bactericidal agent which duplicated the earlier results obtained with the crude protein-containing extract. The present report deals with the toxicity for dogs of a more highly purified protein-free preparation of the bactericidal agent injected by the intravenous route. The

10 Steinbrocker, McEachern, La Motta and Brooks, *J. A. M. A.*, 1940, **114**, 318.
11 Foreign Letter from Paris, *J. A. M. A.*, 1940, **114**, 425.
1 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1; Dubos, R. J., *Ibid.*, 1939, **70**, 11.
2 Dubos, R. J., and Cattaneo, C., *J. Exp. Med.*, 1939, **70**, 249.